Mycorrhizal Infection of Germinating Seedlings of *Nothofagus solandri* var. *cliffortioides* (Hook f.) Poole

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In the course of studying the anatomy of roots of the evergreen tree, *Nothofagus solandri* var. *cliffortioides,* regular observations were made on the natural germination of seedlings in a small tract of forest in the vicinity of the University of Canterbury Mountain Biological Station at Cass. The area concerned is about 2 acres in extent, at an altitude of 2,000 ft. above sea level, isolated in a gully from the main forest areas of *N. solandri* var. *cliffortioides* which dominate the wetter mountainous region a few miles to the west. Rainfall over a 12-month period was 47.9 in. The soil is a mixture of clay and greywacke covered by humus and moss. Growth of the seedlings was inspected at fortnightly intervals over a period of 18 months.

The mycorrhizas of *N. solandri* var. *cliffortioides* are similar in a general way to those of *Fagus sylvatica* (Harley, 1937: 421–423) but are somewhat smaller, and do not give rise to the "intense racemose" type (Harley, 1937: 421–423).

Four months after germination had commenced, a drought of more than 2 weeks was encountered, during which newly formed mycorrhizas shrivelled, many seedlings wilted, and a large proportion died. It was not apparent whether this circumstance had any radical influence on the degree or course of subsequent mycorrhizal infection.

Microscopic examination was made of intact root and mycorrhiza surfaces as well as of serial transections and longisections, in an attempt to follow the pattern of mycorrhizal infection of the seedlings. The present investigation was incidental to anatomical studies of mycorrhizas to be reported elsewhere.

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GERMINATION AND INFECTION

At the time of the emergence of the radicle, in spring, the blackened seed coat was generally enmeshed in mycelial threads of several different types. The testa was intact except at the point of emergence of the radicle, but in a state of superficial decay. In serial microtome sections of the seed, no sign of fungal infection of the expanding embryo was to be seen.

As the radicle grew ahead through moss or leaf mould, hyphae of different colour and form traced rather sparsely over the smooth surface of the root, which maintained a fairly constant diameter of about 0.75 mm. With the appearance of the piliferous layer, however, a more copious growth of fungi took possession of the radicle and became inextricably entangled in the elongating root hairs.

Clamp connections were evident in several of the different types of fungi. Among these was a species constantly present and notable for its long, solitary hyphae, even septation, distinct clamps, and thick golden-brown walls. This fungus appears to have no role in the formation of *N. solandri* var. *cliffortioides* mycorrhizas, although it may be a regular occupant of the rhizosphere of the tree.

Regions of the radicle lacking root hairs were less densely overgrown with fungi than was the piliferous zone.

After 3 or 4 weeks growth of the seedlings (by which time the cotyledons were well expanded), broad sheets of coherent hyphae, in roughly parallel strands, were found connecting the seedling roots with mycorrhizas of nearby trees, from which they had apparently emanated. Most of the fungal wefts joining old mycorrhizas and the root surface of seedlings were white or colourless. In the course of growth, the radicles came also in contact with violet, yellow,

and brown hyphae which nevertheless did not appear to lead to infection or any definite pattern of association.

Radicles in many cases achieved a length of 40 mm. without the appearance of branch roots. Up to this stage, no penetration of roots by hyphae was found though aggregates of mycelium formed in some cases a superficial collar-like layer anterior to the root-hair zone.

Invasion of the radicle had not so far begun.

After 6 or 7 weeks, usually at a time when leaves were expanding above the cotyledons, branch roots became obvious to the naked eye. A considerable proportion grew ahead free of infection, but others were attacked at the tips, as they emerged at the surface, by hyphae massed in a layer along the radicle. Microscopically the laterals so-enveloped by hyphae bore all the characteristics of mycorrhizas. As subsequent laterals reached the root exterior, some were transformed into mycorrhizas and others grew ahead, unimpeded, as nonmycorrhizal roots.

In microtome sections of the radicle, no indication was found of intracellular penetration between mature epidermal cells. In emerging branch roots, intracellular penetration between epidermal cells was found only following radial elongation of the cells of the epidermis. This modification of the epidermal cells appears to be a necessary preliminary condition for penetration by the mycorrhizal fungus. The prior aggregation of mycelium around the root-growing point to form a sheath appears to so alter the development of the epidermis as to enable later penetration.

From the earliest stages of mycorrhiza formation there was a tendency for the root apical meristem of thicker mycorrhizas to burst through the fungal mantle and to continue growth free of infection. Such recovery was not abundant at first but after 3 months became frequent.

By mid-summer, mycorrhizas were present on most seedlings, though most radicles and a proportion of branch roots remained uninfected.

MYCORRHIZAS OF TRANSPLANTED SEEDLINGS

Transplanted mycorrhizal seedlings which were grown out-of-doors in pots in a friable mixture of oak-leaf mould, sand, fine gravel, and loam, continued to form mycorrhizas of a large "simple" type (Harley, 1937: 421–423) over a period of 3 years.

With kitchen garden loams, results were variable. In heavier loams, large uninfected roots predominated. Mycorrhizas were abundant in lighter loams.

In pits of pure river sand, about 2 ft. deep and 3 ft. wide, set in sandy soil, numerous fine and often distorted roots were formed, but mycorrhizas were rare.

When mycorrhizal seedlings were transplanted to pots containing vermiculite watered with a nutrient solution, no mycorrhizas were found after 6 months. The solution used was a basal medium devised by Melin and Nilsson (1950: 89) for mycorrhizal pine seedlings, with glucose and thiamine omitted.

DISCUSSION

It appears that the mycorrhizal association in *N. solandri* var. *cliffortioides* reaches its highest intensity where humus is abundant. In some situations, such as boggy ground and clay, roots generally lack mycorrhizas, and in the case of transplanted seedlings mentioned above the association may be lost, indicating that the balance of interacting growth between fungus and root is quite susceptible to variations in the edaphic conditions.

When the circumstances are favourable, however, it is apparent that massed colonization of the root surface of germinating seedlings is a necessary preliminary stage in the course of mycorrhizal infection. Solitary mycorrhizal hyphae appear not to be able to penetrate the root epidermis.

The presence of sheets of mycelium along the radicle surface results in inhibition in growth of some emerging laterals, accompanied by a radial elongation of developing epidermal cells. Subsequent to this change in the epidermis, hyphae are able to effect intercellular penetration, and a mycorrhiza results. It is possible that inhibition and modification of root growth is due to secretions of auxin by the mycorrhizal fungus (Slankis, 1950: 40–44).

Lack of sufficient "inoculum potential" (Gar-

rett, 1956: 41) of the fungus, or a waning in the secretion of auxin, may account for the recovery from infection of some roots, which are then capable of bursting through the mantle and resuming extended growth.

SUMMARY

The root surface of germinating seedlings of *Nothofagus solandri* var. *cliffortioides* was found to be colonized at an early stage by superficial sheets of mycelium, but intracellular penetration was not apparent until the stage of emergence of lateral roots. Radial elongation of epidermal cells was always found to precede penetration by the mycorrhizal fungus. The presence of massed hyphae over the root-growing point appears to be necessary for the transformation of the root into a mycorrhiza.

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